Approaches for Using Toxicokinetic Information in Assessing Risk to Deployed U.S. Forces

by Karl K. Rozman[1]

ABSTRACT

If there is no exposure, there is no toxicity. If there is exposure, toxicity might ensue when exposure exceeds a certain dose or time, a topic discussed under toxicokinetics and toxicodynamics. Analysis of the fundamental equation of toxicity yields the recognition of three independent time scales. One is the dynamic time scale, which is an intrinsic property of a given compound (what does a chemical do to an organism). The second is the kinetic time scale, which is an intrinsic property of a specific organism (what does an organism do to a chemical). The frequency of exposure denotes the third time scale, which is independent of dose and of the dynamic and kinetic time scales. Frequency of exposure depends on the experimental design or nature, but not on the organism or substance. A liminal condition occurs when the frequency becomes infinite, which corresponds to continuous exposure. Continuous exposure forces the dynamic and kinetic time scales to become synchronized, thereby reducing complexity to three variables: dose, effect, and one time scale. Keeping one of those variables constant allows one to study the other two variables reproducibly under isoeffective, isodosic, or isotemporal conditions. However, any departure from continuous exposure will introduce the full complexity of four independent variables (dose, and the kinetic, dynamic, and frequency time scales) impacting on the effect (dependent variable) at the same time. The examples discussed in this paper demonstrate how nature in the form of long half-lives provides liminal conditions when either kinetic or dynamic half-lives force synchronization of all three time scales.

The original charge for this paper was to conceptualize the role of toxicokinetics in the risk assessment of deployed forces exposed to chemicals. Most toxicologists familiar with current trends in toxicology are aware of the tremendous proliferation of publications combining physiologically based pharmacokinetic (PBPK) models with various dose-response extrapolation models, usually with the linearized multistage (LMS) model, or more recently with the benchmark (BM) curve-fitting approach.

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This author has used both PBPK and classical pharmacokinetics in many experiments. Although both are conceptually sound, there is one fundamental difference: classical pharmacokinetics uses time as an explicit function, whereas PBPK deals with time mostly as a variable, to be predicted based on physiological and physicochemical parameters. Therefore, the concepts of classical pharmacokinetics were helpful in the development of the initial core of a theory of toxicology, as presented in this document, whereas the concepts of PBPK were not as useful. This is not to say that combining PBPK with a theoretically sound biological model will not provide appropriate answers in some instances. However, as long as PBPK is used in conjunction with biologically implausible models (LMS, BM), it will lead (not surprisingly) to insignificant improvements. Central to the development of the concepts presented here was the notion that time is a variable equivalent to dose in toxicology. This idea has been around among toxicologists for almost exactly 100 years. Nevertheless, claims of exceptions to this idea as embodied in Haber's Rule prevented the development of time as a variable of toxicity. Even today toxicologists tend to focus on the so-called "exceptions" when effects are overwhelmingly dose—but not time—dependent. They do not realize that they are studying extreme parts of a spectrum under liminal conditions (e.g., a highly reversible effect on a short time scale), and they use experimental models with insufficient time resolution. When time resolution is satisfactory (such as pungency on a scale of seconds), clear summation effects emerge.

Recognition of the limits of the current risk-assessment paradigm made a paradox clear: none of the current risk projections include time as a variable even though any and all such risk predictions are by definition made in time. From this recognition it was concluded that something that is basically flawed cannot be fixed. Therefore, a new risk-assessment paradigm that includes time as a variable of toxicity, is being suggested. It is clear that although dose is a simple function (number of molecules), time is a complex variable, which runs on many different scales, at least three of which are interacting with dose to provide the complexity that seems to have bewildered generations of toxicologists. The three time scales are the toxicokinetic and toxicodynamic half-lives and the frequency of exposure. Thus, there are three liminal conditions:

1. When the toxicokinetic half-life is very long, it keeps the frequency of exposure essentially infinite (continuous exposure), and the toxicodynamic half-life by definition will be the same as the toxicokinetic one. Under these liminal conditions, $c \times t = k$ for isoeffective experiments, because there is only dose-dependence and one time-dependence.

2. When the toxicodynamic half-life is very long, it requires no additional injury to occur to keep injury constant nor the continuous presence of the noxious agent to result under isoeffective conditions in $c \times t = k$, because there is only dose-dependence and one time-dependence.

3. When the toxicokinetic/toxicodynamic half-lives become very short, they will blur the distinction between the kinetic and dynamic time scales and both will become less important, because in that case the frequency of exposure dominates the time-dependence. Under liminal (continuous exposure = infinite frequency) and isoeffective conditions, this will also lead to $c \times t = k$.

When experiments are conducted under isodosic or isotemporal conditions, then the relationship will obey the equation $c \times t = k \times Effect$. The vast majority of exposure scenarios are of course far from these liminal situations (ideal conditions) and will, therefore, yield $c \times t^x = k$. There are clear suggestions in this paper for the type of experiments that need to be done to determine x with exactitude. In the meantime, practical suggestions are included, which illustrate how to use a decision tree or available databases to conduct risk assessments for deployment situations that are less arbitrary by using both dose and time as variables of toxicity.

The decision tree approach uses a top-to-bottom analysis of identifying rate-determining or rate-

limiting steps in the toxic action of a given compound for a specific effect. The advantage of this approach is its flexibility of determining at what level to contemplate modeling (risk assessment) of toxicity without having to rely on default assumptions. As recognized by other scientific disciplines, understanding of complexity is always advanced at three levels of investigations: experimental, computational, and theoretical. For the most part, toxicologists were and are engaged in experimental and computational studies with very little, if any, progress having been made in developing a comprehensive theory of toxicology. The combined theory and decision-tree analysis presented here should allow rapid progress in improving predictions of toxicity, if experimental design, computational goal, and theory come into equilibrium in terms of checks and balances. Instead of claiming exceptions, the three questions to be asked should be:

1. Why do some experimental results deviate from $c \times t = k$ (isoeffective) or $c \times t = k \times Effect$ (isodosic, isotemporal)?

2. What kind of computational (modeling) approach, and what level of integration, is needed to transform $c \times t^x = k$ or $c \times t^x = k \times Effect$ back to $c \times t = k$ or $c \times t = k \times Effect$?

3. How does exploration of Questions 1 and 2 improve the theory of toxicology, specifically the understanding of k?

It must be recognized that eventually experiments will be conducted under ideal conditions ($c \times t = k$ or $c \times t = k \times Effect$). Once it is known how to transform $c \times t^x = k$ or $c \times t^x = k \times Effect$ (real-life situations) back to the ideal conditions, then any projection will also be possible in the opposite direction. Thus, it can be expected that the vast majority of experiments conducted under less-than-ideal conditions will then become interpretable by using a related study, which has been conducted under ideal conditions.

TOXICITY

Introduction

Toxicity (T) is a function of exposure (E) and E is a function of dose (c) and time (t) ($T = f[E(c,t)]$). Toxicity is the manifestation of an interaction between molecules constituting some form of life and molecules of exogenous chemicals or forms of life affected by physical insults. Consequences of molecular interactions or physical insults might propagate, through causality chains, all the way to the organismic level. There are two fundamental ways to view this interaction: (1) what does an organism do to a chemical, and (2) what does a chemical do to an organism? Dealing with the first question led to the development of the discipline of pharmacokinetics, which was later incorporated into some toxicity studies; in that context it would be more appropriately called toxicokinetics (K). The other question was addressed by the discipline of pharmacology in the form of pharmacodynamic experiments, which again in the context of toxicity, would be more properly termed toxicodynamics (D). Thus, toxicity (T) might be defined as a function of E, K, and D.

$$T = f(E,K,D)$$

A definition of toxicity according to Rozman and Doull (1998) runs as follows "[toxicity] is the accumulation of injury over short or long periods of time, which renders an organism incapable of functioning within the limits of adaptation." This definition implies that toxicity is a function of time in addition to the dose. The latter was already recognized by Paracelsus 500 years ago. A closer scrutiny of the earlier definition of toxicity indicates that the relationship between toxicity, dose (c)

and time (t) is a complex one because toxicokinetics itself is dose- and time-dependent [$K = f(c,t)$] as is toxicodynamics [$D = f(c,t)$]. It is noteworthy that the various time-dependencies seldom run on the same time scale.

Conceptually, K might also be viewed as a function of the dynamic change between absorption (Abs) and elimination (El),

$$K = f(Abs,El)$$

because it is the ratio between entry rate (absorption) and exit rate (elimination) that determines the time course of a compound in an organism. In the simplest case of an iv bolus injection (instantaneous absorption), the time course is determined by the rate of elimination alone for a compound obeying a one-compartment model. Usually absorption is faster than elimination-making processes related to elimination (distribution, biotransformation, excretion) rate-determining or rate-limiting in most instances.

By analogy, D might be viewed as a function of the dynamic change between injury (I) and recovery (R),

$$D = f(I,R)$$

because it is the ratio of injury to recovery that determines the time course of an adverse effect in an organism. The simplest case for such an injury would be when an organism would recover from an acute injury in accordance with a one-compartment toxicodynamic model. Again, processes related to recovery are usually slower than the rate of injury. Therefore, recovery (adaptation, repair, reversibility) will more often be rate-determining or rate-limiting.

Most often compounds do not behave in an organism according to a one-compartment model. The reason for this is that elimination from the systemic circulation itself can be a function of excretion (Ex), distribution ($Dist$), and biotransformation (Bio).

$$El = f(Ex,Dist,Bio)$$

When any or all of these processes become rate-limiting, two- or multi-compartmental models are needed.

Again, by analogy to K, recovery (R) in a D model might not be a simple function of, for example, reversibility (Rv), but could also require repair (Rp). In addition, adaptation (Adp) might also be occurring.

$$R = f(Adp,Rp,Rv)$$

In such instances, two- or multi-compartment toxicodynamic analyses are needed to describe the toxicity of a compound that affects any or all of these processes. Absorption and injury can be thought of as being analogous manifestations of K and D. Absorption is a function of site (S) and mechanism (M) as is injury.

$$Abs = f(S,M)$$
$$I = f(S,M)$$

This analysis can be continued all the way to the molecular level. It is clear that any rate-determining or rate-limiting steps, originating at the level of molecular interactions, will then propagate through causality chain(s) to the levels depicted in Figure 1, which represents a schematic illustration of this concept.

Each of these processes might be dose- and time-dependent, although past experiments often failed to demonstrate this because they were conducted with preponderant emphasis on one or the other variable; for example, D was mainly studied as a function of dose and K mainly as a function of time.

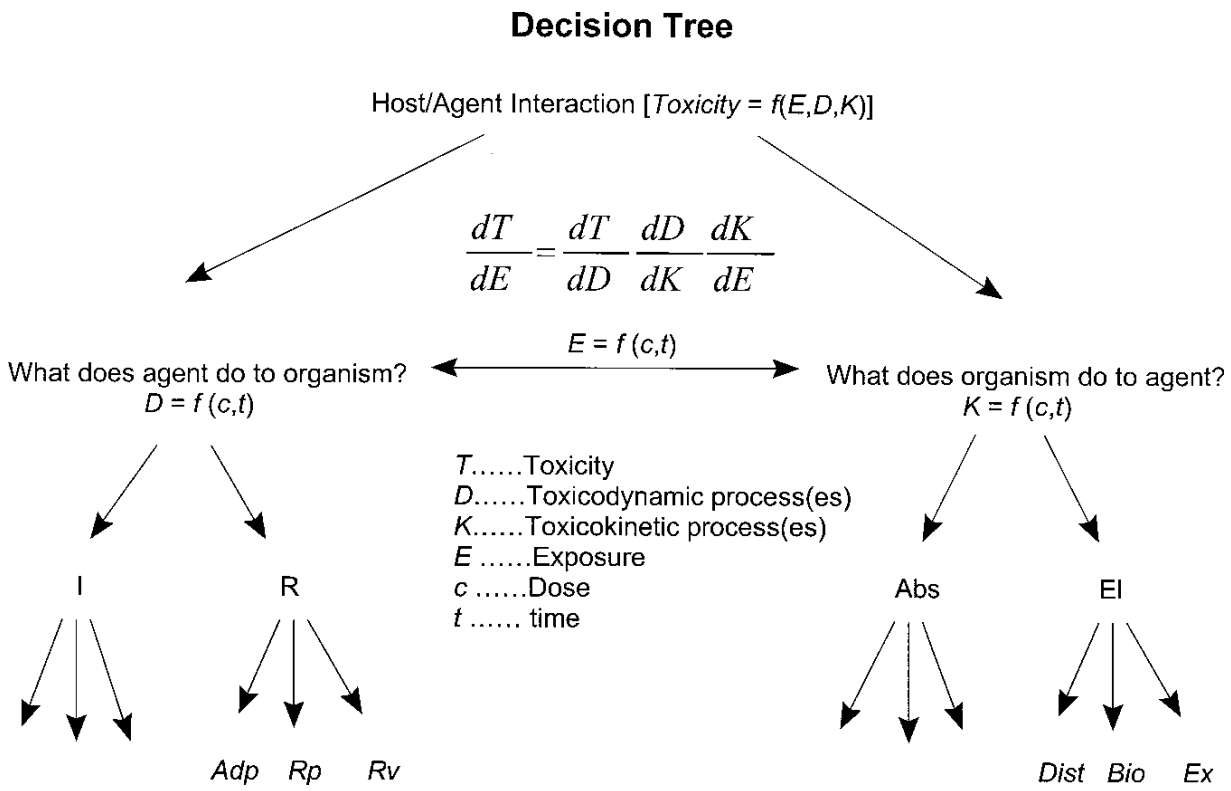

FIGURE 1 Schematic presentation of the decision-tree concept and a mathematical description of toxicity as a function of exposure, kinetics, and dynamics using the chain expansion.

History

Time has always been an important factor in designing toxicological experiments, yet time as an explicit variable of toxicity has been afforded very little attention. It is even more interesting that after Warren (1900) was severely criticized by Ostwald and Dernoscheck (1910) for his analogy of $c \times t = k$ to $P \times V = k$ of ideal gases, the entire issue was forgotten. Even though $c \times t = k$ kept surfacing repeatedly (e.g., Flury and Wirth 1934; Druckrey and Küpfmüller 1948; Littlefield et al. 1980; Peto et al. 1991), an analogy to thermodynamics was not contemplated again, at least not to this author's knowledge! With the "rediscovery" of the $c \times t = k$ concept in still another context (delayed acute oral toxicity), some reevaluation regarding the role of time in toxicology in a historical context is required.

Ostwald and Dernoscheck's (1910) analogy of toxicity to an adsorption isotherm is problematic, because adsorption entails processes far from ideal conditions. Much more reasonable is Warren's (1900) analogy to $P \times V = k$ for ideal gases as a comparison to ideal conditions in toxicology. Reducing the volume of a gas chamber containing a given number of molecules or atoms of an ideal gas will decrease the time for any given molecules or atoms to collide with the wall of the chamber and will lead to increased pressure, which is simply an attribute of the increased number of molecules per unit volume, which is concentration. Thus, $c \times t = k$ and $P \times V = k$ are compatible with each other if looked at mechanistically. Of course, Ostwald and Dernoscheck's comparison of toxicity to an adsorption isotherm is much closer to the real-life situation of toxicology where the most frequent finding is that of $c \times t^x = k$.

These thought experiments and some discussions led to the recognition that toxicologists did everything the opposite of what thermodynamicists did. Instead of starting out with the simplest model (ideal gas in thermodynamics corresponds to ideal conditions in toxicological experiments) and building into it step by step the increasing complexity of the real world, toxicologists tried to predict from one complex situation to another complex situation. In addition, time was largely ignored, although it is one of two fundamental variables of toxicity (Rozman 1998). It is unlikely that a better understanding of biological processes at the molecular level alone will lead to improved risk predictions in toxicology, as long as the experimental designs of toxicological studies provide the wrong reference points for departure from ideal to real conditions. For example, the standard inhalation toxicity protocols (6 h/d for 5 d/wk) cannot yield $c \times t = k$ because after 6 h intoxication, there are up to 18 h of recovery, and on weekends there are up to 66 h of recovery, at least for compounds of short half-life. This would require at least two additional functions to correct for departure from steady state. The real-life situation is even more complex when departures from the ideal condition (steady state) are highly irregular. Nevertheless, it is reasonable to expect that risk predictions will be possible for even the most irregular exposure scenarios once the reference points are established as dose- and time-responses under ideal conditions (toxicodynamic or toxicokinetic/toxicodynamic steady state) and then departures of increasing complexity are defined.

In 25 years of studying the toxicity of tetrachlorodibenzodioxin (TCDD) and related compounds, the concept of $c \times t = k$ did not emerge in any other experimental context except in the two recent subchronic and chronic toxicity studies, which were conducted under conditions of toxicokinetic steady state (Rozman et al. 1996; Viluksela et al. 1997, 1998). Nevertheless, a general interest in the role of time in toxicology pervaded the line of thinking presented here for many years (Rozman et al. 1993; Rozman et al. 1996; Rozman and Doull 1998; Rozman 1998). Most toxicologists are familiar with Haber's Rule of inhalation toxicology and its applicability to war gases and some solvents. Much less reference has been made to Druckrey's work (Druckrey and Küpfmüller 1948, Druckrey et al. 1963, 1964, 1967), which extended the $c \times t$ concept to lifetime cancer studies by oral rather than inhalation exposure. And finally, there is very little cross-referencing of the $c \times t = k$ data, which were generated by entomologists (e.g., Peters and Ganter 1935; Busvine 1938; Bliss 1940) and those established by toxicologists. History demonstrates that a fundamental relationship in science keeps reappearing in different contexts, as is the case with $c \times t = k$. During this period many apparent exceptions seem to be occurring with no satisfactory explanation. Attempts at generalization usually fail until a commonality is detected among all experiments, as is the case among those that yielded $c \times t = k$. This commonality is toxicokinetic steady state or irreversibility of an effect, which of course can be interrelated. Anesthesia, like intravenous infusion, leads to rapid and sustained steady state for compounds of short half-life. Most anesthetics and solvents do have short half-lives and many obey Haber's Rule, except when measurements are taken while an adaptive process is under way, that is, induction of a protein. Druckrey and the ED_{01}-Study used feeding as a route of exposure, which yields a better steady state for compounds of intermediate half-life than, for example, gavage. However, the exponent x in the term of Druckrey's general formula increases above one rapidly as the half-life of compounds becomes shorter, because there is intermittent recovery between bouts of feeding. Most of the entomology studies were related to fumigation, which often but not always resulted in fairly rapid steady state. And finally 1,2,3,4,5,6,7,8-heptachlorodibenzo-p-dioxin (HpCDD), which has a half-life of 314 days (Viluksela et al. 1997) in female rats yields virtual steady state for a 70-d observation period after any route of administration but not TCDD with a half-life of 20 d. However, when TCDD's toxicity was studied under steady-state conditions, its subchronic and chronic toxicity also occurred according to $c \times t = k$ (Rozman et al. 1993).

DOSE AND TIME AS VARIABLES OF TOXICITY

Definition of Dose and Time

Before analyzing dose and time relationships further, it is useful to come up with clear definitions of these fundamental variables of toxicity. Due to historical developments, neither dose nor time has been defined with clarity as variables of both toxicokinetics and toxicodynamics. It is customary to use the term acute dose and acute effect as if the two were interchangeable. In fact, an acute dose can lead to chronic effects (Druckrey et al. 1964) and multiple doses can trigger a fulminant episode of toxicity (Garrettson 1983). In risk (safety) assessment it is always the total dose delivered that is of concern, although in therapeutics the daily dose is often referred to simply as the dose. Therefore, a useful definition of dose in toxicology, would be:

$$Dose = \sum_{n=1}^{n} Dose\ Rates$$

According to this definition a single acute dose would represent the liminal case when the dose rate equals the dose. This definition would be valid for any kind of irregularity in the dosing regimens and is analogous to the definition of dose in radiation biology.

Ever since the dawn of human consciousness, humanity has struggled with the notion of time. It is not possible to predict what influence the concept of toxicological time will have on our perception of time. Suffice it to say at this junction, it is not possible to think of toxicty without the implicit presence of time as a variable, although in toxicological studies, time received only semiquantitative designations (acute, subacute, subchronic, chronic). In fact, one could view organisms as instruments exquisitely sensitive to time. Important for toxicology is that the time course of a toxicant in an organism (kinetics) is very often different than the time course of toxicity (dynamics). Underlying biological processes (absorption, distribution, elimination, injury, adaptation, recovery) have their own time scales, depending on the molecular events behind each process (e.g., enzyme induction, receptor regulation either directly or via gene expression). Thus, in toxicology the dose is a pure variable, but there are many different processes occurring on different time scales yielding different $\int c dt$ integrals leading to complex interactions, which can be described as $c \times t^x$. In spite of this complexity, science can deal with it in a traditional, analytical fashion. Because only knowledge of rate-limiting steps is required to accurately describe toxicity, this will often reduce complexity to manageable proportions.

Dose and Time Relationships

Consequences of interactions between a toxic agent and an organism at the molecular level propagate through toxicodynamic or toxicokinetic/toxicodynamic causality chains all the way to the manifestation of toxicity at the organismic level (Figure 1). If the recovery (adaptation, repair, and reversibility) half-life of an organism is longer than the half-life of the causative agent in the organism, then toxicodynamics become rate-determining (one-compartment model) or rate-limiting (multi-compartment model). If the toxicokinetic half-life of the compound is longer than the recovery half-life, then toxicokinetics will be rate-determining (rate-limiting), in which case the toxicokinetic area under the curve (AUC) will be identical to the toxicodynamic AUC. There are three liminal conditions for $c \times t = k$ that emerge when the causality chain propagates through either toxicodynamic or toxicokinetic/toxicodynamic processes:

Toxicodynamics

1. In case of no recovery (no reversibility, no repair, no adaptation) linear accumulation of injury will occur according to a triangular geometry ($c \times t/2 = k$) following repeated doses or according to a rectangular geometry after a single dose ($c \times t = k$), provided that the $c \times t$ lifetime threshold has been exceeded, which occurs when $c_{\text{threshold}} \times t_{\text{lifespan}} = k$.

2. After recovery (reversibility, repair, adaptation) steady state has been reached, injury will occur according to a rectangular geometry ($c \times t = k$), after exceeding the $c \times t$ lifetime threshold.

Toxicokinetics

1. No elimination will lead to linear accumulation of a compound and, as a consequence, to accumulation of injury according to a triangular geometry ($c \times t/2 = k$) after repeated doses or according to rectangular geometry after a single dose ($c \times t = k$) above the $c \times t$ lifetime threshold.

2. After toxicokinetic (and as a consequence toxicodynamic) steady state has been reached, injury will occur above the $c \times t$ lifetime threshold according to a rectangular geometry ($c \times t = k$).

Exposure Frequency

As the toxicokinetic and toxicodynamic half-lives become shorter and shorter, the distinction between elimination and recovery half-lives becomes less important, because another time-dependence, that of the frequency of exposure, starts dominating the time-dependence.

1. Compounds having very short toxicokinetic/toxicodynamic half-lives will reach steady state rapidly and yield $c \times t = k$ upon continuous exposure according to a rectangular geometry, provided that adaptation and repair are also at steady state.

2. Other types of geometries certainly can be created by elaborate, but regular, dosing regimens. These scenarios are less likely to play a practical role in toxicology, although they might be of theoretical interest.

It should be kept in mind that the mathematics of first-order processes, when appropriate, are valid for bi-molecular reactions (e.g., receptor binding), which result in the propagation of the causality chain to the level of modeling (Figure 1). Therefore, 90% of toxicodynamic steady state will not be reached until 3.32 recovery half-lives have elapsed. Thus, Haber's Rule will be obeyed only if the observation period is outside of about 4 recovery half-lives or if recovery is a zero order process.

Thus, the various ($c \times t = k$) scenarios represent liminal conditions. The magnitude of the $c \times t$ product is a function of the potency of the compound, of the susceptibility of the organism, and of the deviation from the ideal conditions and will yield $c \times t^x = k$ for nonliminal conditions. (Large $c \times t^x$ product indicates either low potency, lack of susceptibility, or low exposure frequency.) It must be recognized that the dose *(c)* does not have exponential properties, but time *(t)* does have such properties, because under nonideal conditions toxicity is a function of at least two independent time scales. One independent time scale is the half-life of the rate-determining step (toxicodynamic or toxicodynamic/toxicokinetic) of the intoxication (intrinsic property of compound or organism), the other one is the frequency and duration of exposure, which is independent of both the compound and the organism.

In conclusion, these data and considerations of a significant body of evidence accumulated over the last 100 years suggest that $c \times t = k$ is a fundamental law of toxicology, and possibly of biology in general, that can be seen only under ideal conditions. If confirmed using other classes of compounds

and the ideal conditions described here, then Paracelsus' famous statement might have to be supplemented to read *Dosis et tempus fiunt (faciunt) venenum* (Dose and time together make the poison). Implications for risk assessment are that the margin of exposure (MOE) must be defined in terms of both dose and time. This can be done by relating the real-life exposure scenario to that of the ideal exposure condition:

$$MOE = \frac{c \times t^{x}}{c \times t}$$

Above the $c \times t$ lifetime threshold, this will yield the margin of safety and its reciprocal, the margin of risk.

Figure 1 might also be viewed as a decision tree to identify critical steps needed for modeling to predict toxicity. It is important to note that a high degree of irreversibility and toxicokinetic steady state are rare phenomena in toxicology, although both can be seen any time when the observation period is much shorter than the recovery or the elimination half-life. In the real-life situation, there are usually at least two or three rate-limiting steps in toxicokinetics and likely as many in toxicodynamics. It must be emphasized though, that multiple toxicokinetic compartmental models do not necessarily require multiple toxicodynamic models, and vice versa. However, if there are three different rate-limiting processes occurring on different time scales in toxicokinetics and three different rate-limiting processes taking place on three different time scales in toxicodynamics, such a scenario would represent a formidable computational task for a theoretical treatise. Therefore, a practical approach would be to conduct experiments at toxicodynamic steady state (which of course would require a preexisting toxicokinetic steady state in many instances) as a point of reference clearly defined by $c \times t = k$. Then, experiments need to be carried out for different compounds with different half-lives to establish model parameters, which describe departures from toxicokinetic/toxicodynamic steady state of increasing frequency and irregularity.

In summary, $c \times t = k$ represents the most efficient (a kind of worst-case) exposure scenario for producing an effect, namely continuous exposure until manifestation of an effect. Experimentally, this condition is often met by continuous inhalation exposure (e.g., Gardner et al. 1977), or daily oral administration of compounds that have toxicodynamic/toxicokinetic half-lives of a few days or longer or effects that are essentially irreversible. It must be emphasized that any departure from the worst-case scenario will result in a change of $c \times t = k$ into $c \times t^{x} = k$. Departures are represented by regular or irregular interruptions of exposure or intermittent recovery from injury. The larger the departure, the larger will be x, and with that k. It is somewhat counterintuitive, but increasing x and k are equivalent to decreasing toxicity. This is entirely logical, however, when it is recognized that increasing interruptions of exposure or injury will result in longer and longer periods of time needed to cause equivalent toxicity to that of continuous exposure, because of increasing intermittent recovery. A liminal condition for first-order processes will be reached when exposure occurs outside of 6.6 toxicokinetic/toxicodynamic half-lives, because at that time 99% elimination or recovery will have occurred. Under such conditions (which are closest to the real-life situation for most compounds), toxicity will be less dose-dependent and toxicokinetic/toxicodynamic time-dependent, and mainly the frequency of exposure will determine x. If x is then determined experimentally, for example, for 1, 2, 4, 8, 16, and 32 days for a compound with a toxicokinetic/toxicodynamic half-life < 3.6 h after continuous versus intermittent exposure under isoeffective conditions, then plotting the data will allow extrapolation to any exposure scenario outside of 6.64 half-lives (which corresponds to 1 day). Most dietary constituents fall in this category. For zero-order processes, 2 half-lives are needed for elimination or recovery. It should be kept in mind that the half-life of zero-order processes (unlike that of first-order processes) is dependent on concentration.

Analogy to Thermodynamics

In physics, Boyle's Law of ideal gases, gave rise to thermodynamics, and molecular and mechanistic considerations led to a theory of gas reactions. The former is based on the idea of finding the minimum number of fundamental variables that can describe the simplest possible dynamic system ($P \times V = k$ for ideal gases). The latter required a great deal of knowledge about the mechanism of chemical reactions, such as wall reaction and activation energy. Both of these approaches have been attempted in toxicology with, as yet, limited success, as shown in subsequent discussions in this paper. The reason for the lack of advance in theoretical toxicology is probably due to the fact that, unlike thermodynamicists, toxicologists did not start out with defining the simplest possible toxicological conditions with a minimum number of variables as a point of departure toward more complexity. Coincidentally, experiments were conducted under such ideal conditions and in every such instance Haber's Rule proved to be applicable (Gardner et al. 1977), even though researchers might have failed to notice it (Sivam et al. 1984).

The lack of conceptualization of the three variables of toxicity resulted in arbitrary study designs, which further eroded the predictability from one experiment to another. It is this author's opinion that analogous thinking to thermodynamics might help to optimize study design and eventually to build a theory of toxicology. Thermodynamics, like toxicology, has three fundamental variables (*P,V,* and *T* versus *c*, *t*, and *W*). (*W* [German for Wirkung]) will be used for effect, because of the many *E*s ([exposure, elimination, effect, excretion] in English.) Before the development of a comprehensive theory of thermodynamics, it was clear to scientists that to study an independent and a dependent variable, a third or other variables had to be kept constant. This has not been done in toxicology, although most dose-response studies have been conducted at constant time (isotemporal). However, to study the relationship between time and effect, the dose needs to be kept constant (isodosic). Moreover, to examine the relationship between dose and time, the effect must be kept constant (isoeffective). The $c \times t$ product will not emerge from the equation of ergodynamics (Figure 2) until after elucidation of the relationship between specific effect at constant time and specific effect at constant dose. In other words, more must be learned about *k* before significant theoretical advance is possible. As mentioned before, most experiments have been conducted isotemporally in the past (14 d, 90 d, 104 wk), which is appropriate for dose-response studies. The arbitrary choice of these time points and the inexactitude of diagnosis (stuff them and count them) led to a great deal of confusion in the 14-day studies, because different dose responses with different mechanisms were often lumped together. Experiments in toxicology have frequently been conducted under isoeffective conditions, mainly with the endpoint being 100% of an effect (mortality, cancer). However, systematic investigation of $c \times t = k$ has not been done, for example, at 20 or 80% of an effect. Finally, there have been very few experiments conducted under isodosic conditions, because this requires that the concentration be kept constant at the site of action. The only experiment-driven condition when this is often the case is inhalation exposure. Gardner et al. (1977) have reported such data after continuous exposure of experimental animals to benzene and SO_2 when the endpoint in question was measured immediately after termination of exposure (chronaxy, leukopenia). However, when the endpoint of measurement was not immediately done (streptococcal infection-related mortality) after cessation of NO_2 exposure, the time response started flattening out (Gardner et al. 1979). A systematic investigation of these issues has been done recently for HpCDD after oral administration with as yet only one endpoint of toxicity (delayed acute toxicity), although preliminary analysis indicates that there are other valid endpoints such as anemia and lung cancer (Rozman 1999). These data provide support for the suggestion of Rozman et al. (1996) that the dose-time-response be viewed as a 3-dimensional surface area similar to, but conceptually distinctly different from, the model of Hartung (1987) (Figure 3). Experiments conducted under isoeffective conditions

ERGODYNAMICS

$$dW = \left(\frac{\partial W}{\partial c}\right)_t dc + \left(\frac{\partial W}{\partial t}\right)_c dt$$

$$-\left(\frac{\partial W}{\partial c}\right)_t dc = \left(\frac{\partial W}{\partial t}\right)_c dt$$

$dW = 0$ isoeffective

$$dW = \left(\frac{\partial W}{\partial c}\right)_t dc$$

$$dW = \left(\frac{\partial W}{\partial t}\right)_c dt$$

$dt = 0$ isotemporal

$dc = 0$ isodosic

FIGURE 2 Definition of toxicity in analogy to thermodynamics.

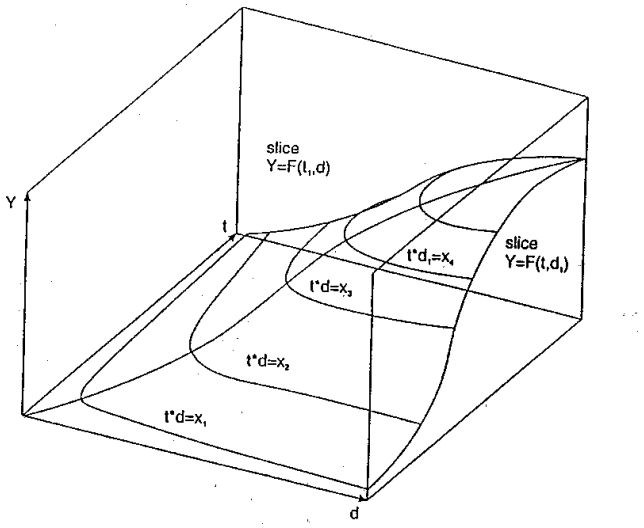

FIGURE 3 Schematic presentation of the dose-time-response surface area showing slices for isoeffective (hyperbolas), isodosic (S-shaped time response), and isochronic (S-shaped dose response) responses.

(slices parallel to the dose-time plane) correspond to Haber's Rule of $c \times t = k$ represented by hyperbolas. Studies carried out under isotemporal conditions (slices parallel to the time-effect plane) yield S-shaped dose-response curves along which $c \times t = k \times W$ whereas isodosic investigations (slices parallel to the dose-effect plane) produce S-shaped time-response curves along which $c \times t = k \times W$ also. Indeed, plotting the $c \times t$ product against effect (W) for HpCDD for doses causing about 10 to 90% wasting/hemorrhage, yielded a straight line of high correlation ($r^2 = 0.96$) (Figure 4). This is the beginning core of a theory of toxicology, which is analogous to $P \times V = k$ for isotherms, and $P \times V = k \times T$ for isobars or isochors. Of course, thermodynamicists know that $k = n \times R$ (n = number of moles; R = gas constant), but toxicology is not yet there. What is clear already at this junction is that the dimension of $P \times V$ is energy, whereas the dimension of $c \times t$ is energy × time, which is action and is called effect in toxicology (Figure 5).

DECISION TREE

A recent series of articles explored how other disciplines deal with complex systems (Goldenfeld and Kandanoff 1999; Whitesides and Ismagilov 1999; Weng et al. 1999; Koch and Laurent 1999). Goldenfeld and Kandanoff (1999) made some important observations, that are relevant for toxicology. Simple laws of physics give rise to enormous complexity when the number of actors is very large. The same paradox exists in toxicology in that the $c \times t$ concept is very simple, but the "real world" of the manifestation of toxicity is very complicated. Their other observation is equally relevant; "Use the right

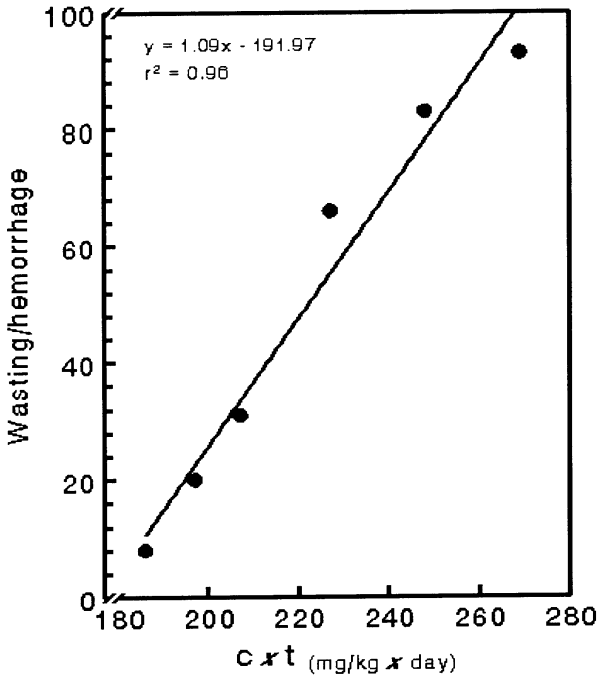

FIGURE 4 A $c \times t$ wasting/hemorrhage plot in HpCDD-treated rats. Data were taken from Rozman (1999) and the second lowest dose added.

Ergodynamics:

$$c \, x \, t = k \, x \, W$$

***W*.....Effect (Wirkung)**
***c*.....dose**
***t*..... time**

Thermodynamics:

$$P \, x \, V = n \, x \, R \, x \, T$$

$$dW = \left(\frac{\partial W}{\partial c}\right)_t dc + \left(\frac{\partial W}{\partial t}\right)_c dt$$

Physics:

Action = Energy *x* Time

Toxicology:

***W* = Dose *x* Time**

Dose is a form of energy.

FIGURE 5 Additional analogies between thermodynamics and ergodynamics.

level of description to catch the phenomena of interest. Don't model bulldozers with quarks." The decision tree (Figure 1) was developed to aid toxicologists and modelers to identify both the appropriate phenomena and the right level of modeling. Toxicologists can avoid much unnecessary experimentation by using this top to bottom approach rather than the currently fashionable bottom to top approach.

Toxicokinetics [$K = f(Abs, El)$]

This discipline deals with the mathematical modeling of the question, "What does an organism do to a chemical?" Pharmacokinetics is, and was, dominated by investigations of the time course of drugs and other chemicals in humans and in animals. Dose-dependence was seldom studied, except when a saturation phenomenon resulted in some unexpected data. This is understandable, because the therapeutic range is in the (hormetic) dose region of adaptation or improved repair and not in the toxic range. However, toxicity (such as body weight loss or renal or liver damage) is certain to alter the kinetics of the toxicant or of other toxicants, as has been demonstrated repeatedly (e.g., Weber et al. 1993; Roth et al. 1994; El-Masri et al. 1996). Procedurally, toxicokinetics needs to be determined at a nontoxic and a highly toxic dose. It would be advisable to do the kinetics right after a toxicological dose-range finding. Ideally, the kinetics should also be determined in humans at a nontoxic dose. Early determination of toxicokinetics is very important, because the design of toxicodynamic experiments might be critically dependent on the half-life of a compound and on other kinetic information (Figure 1).

For analytical purposes, toxicokinetics can be divided into separate considerations of absorption and

elimination (biotransformation, distribution, and excretion). Although they are overlapping, each of these processes has its own dose- and time-dependence. The notion of AUC, which is the integration of dose over time, is well recognized in *K*.

Absorption [$Abs = f(S,M)$]

There are three main entries for chemicals into the body: oral, inhalation, and dermal. There are a number of minor portals of entry, such as the eyes and the nasal mucosa, as well as artificially created ones like intramuscular, subcutaneous, intravenous, and intraperitoneal. Oral absorption is by far the most common route of exposure to chemicals. It should be kept in mind that this route of exposure is well controlled by the food supply of highly developed countries, but not so in less developed nations. Inhalation and dermal exposure to chemicals is usually an involuntary process with varying contributions to total exposure. In general, absorption is much less likely to be a rate-determining or rate-limiting step than elimination is.

Oral Absorption

This process is dose- and time-dependent. Dose-dependence is usually due to saturation phenomena (active transport, facilitated diffusion, limited solubility). Time-dependence of absorption is linked to the dynamism of the gut (passage of ingested material). Determination of the time course of absorption is standard practice in pharmacokinetic studies of drugs. In toxicological studies, measurement of oral absorption is, unfortunately, not standard practice. But, it is not fundamentally different from the absorption of drugs, and it has been done often enough to arrive at generalizations (Aungst and Shen 1986; Rozman and Klaassen 1996). The time course of oral absorption is limited by the dynamism of the gastrointestinal (GI) tract and the physicochemical properties of the chemicals being absorbed. Absorption of amphophilic chemicals is quite efficient from the hydrophilic contents of the gut. Highly hydrophilic chemicals, such as metal ions, penetrate the GI tract to a very limited extent because their large hydration shells prevent movement with bulk flow of water through aqueous pores. Often there are active transport processes to carry such molecules across membranes, but transporters are usually saturated at low concentrations. Highly lipophilic chemicals are absorbed in the context of lipid digestion (e.g., micelles and chilomicrons) via the lymphatics. The residency time of the GI contents sets a limit for absorption. Contents move rapidly through the duodenum and jejunoileum, traversing about half of the small intestine in about 20 min after leaving the stomach. They reach the ileocecal valve after about 1 h. Contents remain for up to 36 h in the large intestine, being churned there by peristalsis and antiperistalsis, but absorption of chemicals other than water is limited in this part of the GI tract. Oral absorption entails chemicals entering the systemic circulation after being transported to the liver by the portal vein. Most compounds will be detoxified, in the liver, to more readily excretable metabolites. Metabolic activation and subsequent hepatotoxicity must be considered, although this is the exception. Allometric species differences must be taken into account when animal data are interpreted for humans.

This discussion illustrates that oral absorption of chemicals can be well managed during other than customary circumstances of short- or long-term exposure. The critical factor is control of the food and drinking water supplies, which determine the dose-dependence (source, size of meals and drinks) and time-dependence (frequency of meals, duration of exposure) of oral absorption. Ingestion of particulate matter (dust, sand, and aerosol) is of low concern, because exposure by inhalation will be the major route of their uptake. The best way to protect deployed forces from potential oral exposure to toxicants is to provision them with food and water from the home country by meals ready to eat (MRE), until the safety of local resources has been ascertained.

Inhalation

Due to the enormous surface area of the lungs, absorption here can be much more efficient than in the GI tract, particularly for volatile compounds. There are no special transport processes for ions or lipids in the lungs, with corresponding consequences for their absorption by inhalation. For a more detailed discussion of absorption by inhalation, see Casarett and Doull's Toxicology (Rozman and Klaassen 1996). For purposes of this paper, it is important to consider at what point absorption by inhalation will represent a rate-determining or rate-limiting step on the decision tree (Figure 1). Absorption by inhalation is either ventilation (high solubility in blood) or blood-flow-limited (low solubility in blood). In either case, absorption in the lungs is almost as rapid as intravenous infusion, which means that it is rarely, if ever, rate-determining. Nevertheless, it should be kept in mind that, as with iv infusion, 99% of steady state will be reached by inhalation after about 7 elimination half-lives.

Protection of deployed forces from exposure to toxicants by inhalation is the most difficult task. Defense against war gases utilizes a combination of a prophylactic antidote administration and gas masks. However, this is not a topic here, although interesting kinetic considerations are also applicable in that context. Inhalation exposure will become rate-determining only for compounds with low lung clearance such as silica, asbestos, dusts of various kinds, and soot. Due to their low clearance, they tend to accumulate in the lungs and cause mechanical injury or sustained immunoresponse. Exposure to these agents is often unavoidable, because it is part of the deployment conditions. According to considerations depicted in Figure 1, the toxicity of such substances will be determined by toxicokinetics and, hence, by the toxicokinetic/toxicodynamic AUC generated, which implies that it will occur according to $c \times t = k$ above the lifetime threshold. A deviation from 1 as exponent on t will depend on the low lung clearance, although such deviation is expected to be minor. A practical way to determine the risk (safety) of such substances for various lengths of deployment is to identify appropriate standards of the Occupational Safety and Health Administration (OSHA), the American Council of Governmental Industrial Hygienists (ACGIH), or the Environmental Protection Agency (EPA) and calculate a $c \times t$ for a particular deployment situation. For example, ACGIH recommends a no-observed-adverse-effect level (NOAEL) of 0.1 fiber/cm^3 of asbestos for a 45-yr working life, at 8-h/d, 5 d/wk.

Amount of air inhaled in 8 h: 10 m^3
Amount of fibers inhaled in 45 yr: 0.1×10^6 fibers/m^3 (cm$^3 \rightarrow$ m^3) $\times$ 10 m^3 (air inhaled) $\times$ 5 d/wk $\times$ 52 wk $\times$ 45 yrs = 1.17×10^{10} fibers
Amount that might be inhaled in 14 d: 1.17×10^{10} fibers
In one day: $1.17 \times 10^{10}/14 = 8.357 \times 10^8$ fibers/d
Amount of air inhaled in 24 h: 20 m^3
$8.357 \times 10^8/20$ m^3 = 4.178×10^7 fibers/m^3, or $4.178 \times 10^7/1 \times 10^6$ cm^3 = 41.8 fibers/cm^3
14-day deployment exposure limit (DEL): 41.8 fibers/cm^3

It is a risk-management decision how much reserve capacity needs to be retained for the remaining life expectancy. This example illustrates that a simple calculation, by using the $c \times t$ concept when appropriate, will yield a safe level of exposure for any length of deployment.

Dermal Absorption

The skin is an effective barrier separating higher organisms from their environment. The rate-determining process in dermal absorption is the stratum corneum, which is a dead keratinized cell layer, composed mainly of ceramides. Physicochemical properties of molecules determine whether or not a particular compound will or will not (and to what extent) penetrate this barrier. Highly water-soluble

and highly lipid-soluble compounds have the poorest penetration rates, whereas compounds most similar to ceramides have the highest penetration rates. For more detail, see Cassarett and Doull's Toxicology (Rozman and Klaassen 1996). For conceptual purposes, unlike the GI-tract and the lungs, dermal penetration can be rate-limiting in the toxicokinetics of compounds, because elimination of substances by biotransformantion (often a first-order process) and excretion are often more rapid than the rate of dermal absorption (often a zero-order process). For these reasons, systemic effects after dermal exposure are not as frequent as generally believed. The most frequent toxicodynamic sequel of dermal exposure is local irritation or allergic reaction, for which exposure itself is rate-determining. For systemic considerations, dermal absorption will be critical only if it results in accumulation of injury, as in the case of soman, or if a large surface area is exposed to a small, rapidly penetrating molecule like chloroacetic acid.

Considering that deployed forces will be wearing uniforms most of the time when dermal exposure to toxicants might be occurring, the only sites of exposure will be the hands and facial area. Although penetration rates of chemicals are variable, they are seldom faster than a few $\mu g/cm^2$. Therefore, dermal exposure under usual deployment conditions will have few or no toxicological consequences for the vast majority of toxicants, with the notable exceptions of irritants and allergens.

Elimination [*El = f(Ex,Dist,Bio)*]

Elimination terminates the interaction between molecules of an organism and molecules that invaded it. It is most often the rate-determining step in the dynamic equilibrium between absorption and elimination. Therefore, in instances when toxicokinetics determines the toxicodynamic process or processes, this is the step that requires modeling. Elimination means removal of a compound from the systemic circulation. This can be due to distribution, sequestration in non-target tissues, biotransformation, or excretion.

Excretion

This process ends the presence of a chemical in an organism. In the simplest case of an iv bolus injection of a toxicant, if the compound is excreted mainly by urinary excretion, one would obtain a straight line (one-compartment model), on a log C_p versus time plot. For chemicals that are very rapidly cleared, excretion will not be rate-determining, because biotransformation is usually a slower process, and hence it will dominate the overall time course of elimination.

Renal Excretion. This is most often the main route of excretion. It is well understood, including the consequences of its impairment in the pharmacokinetics of drugs (Gibaldi and Perrier 1975). It will seldom represent the rate-determining or rate-limiting step in the toxicodynamic action of chemicals, because of its rapidity. Modeling of urinary excretion of toxicants will be useful only if there is no other rate-limiting step in the disposition of a compound.

Fecal Excretion. Bile and direct transfer across the intestinal mucosa are the two major sources of toxicants in feces. Both processes are reasonably well understood (Gregus and Klaassen 1986; Rozman 1986). After hepatic biotransformation, biliary excretion is usually fast and conjugates will be rehydrolyzed in the GI tract, leading to reabsorption and often followed by urinary excretion. A small fraction might escape hydrolysis and be excreted in feces. The half-life of a compound excreted with bile with or without enterohepatic circulation can be, nevertheless, quite short. The major route of excretion of chemicals that are resistant to biotransformation is also fecal excretion. Important for risk assessment is

when fecal excretion represents the slowest step in the disposition of toxicants and, at the same time, toxicokinetics represents the rate-determining step in the toxicodynamic action of a compound. This is the case for the infamous class of compounds generically called "dioxins." Half-lives of chemicals become very long when biotransformation is highly inefficient and excretion occurs primarily by nonbiliary intestinal processes, such as desquamation of intestinal epithelium or direct diffusion from the capillary bed into the GI contents. Both are slow processes, exfoliation being limited by the proliferation of the intestinal mucosa and intestinal excretion being limited by the small volume of distribution of the GI contents and their slow flow rate. Interestingly, in spite of their supposed unimportance, these processes can dominate the toxicodynamics of certain classes of chemicals, whereas urinary excretion as the major route of elimination much less frequently determines toxicodynamics.

Biotransformation

This is perhaps the most extensively studied part of the elimination process of chemicals (e.g., Hodgson et al. 1991; Parkinson 1996). Rightly so, because it probably represents, most frequently, the rate-limiting step in the elimination of chemicals from the body. Therefore, in instances when toxicokinetics is responsible for a given toxicodynamic AUC, biotransformation requires careful evaluation. It is not the purpose of this paper to review any part of the vast literature on Phase I and Phase II biotransformations. Rather, the aim is to outline under what conditions biotransformation becomes rate-determining or rate-limiting. Most often, biotransformation is a process of recovery from a toxic insult, because both Phase I and more so, Phase II metabolism leads to more water-soluble, and hence more readily excretable, metabolites. Sometimes, however, biotransformation leads to metabolic activation, that is, to more toxic derivatives. If toxicodynamics rather than toxicokinetics is rate-determining then metabolic activation is of no consequence, because the dynamics of recovery from the lesion will dominate the time course of the effect. For example, an enormous amount of effort has gone into understanding the mechanism of toxicity of benzene in terms of metabolic activation. However, the half-life of benzene is about 8 h in humans and not much different in animals (Brugnone et al. 1992; ATSDR 1997), whereas the hematopoietic system replenishes erythrocytes on a time scale of about 120 days (e.g., Winthrobe and Lee 1974). In equilibrium, this must be the rate of maturation of erythrocytes from stem cells. The propagation of any lesion at any step of this process will be subject to this time scale. Therefore, the dynamics of benzene toxicity will be dominated by the dynamics of the lesion and not by kinetics. Consequently, risk assessment of benzene exposure should be driven by the frequency of exposure above the lifetime threshold and not by some low-level continuous environmental exposure. According to these considerations, once a month exposure to very high concentrations of benzene would result in accumulation of residual damage (according to $c \times t$ after reaching steady state), until the individual's aplastic anemia (or leukemia on still another time scale) threshold has been exceeded. Therefore, preventive measures regarding benzene toxicity should focus on reducing peak concentrations and the frequency of exposure (ideally peak-exposure frequency should be reduced to less than once in 100 days). If safety considerations were to be based erroneously on toxicokinetics, the conclusion would be that continuous exposure to lower levels of benzene would be more dangerous (larger AUC) than intermittent exposures to high-peak concentrations. In any event, understanding of the mechanism of action of a chemical helps to identify the rate-determining or rate-limiting step (or steps), because the dose- and time-responses ($c \times t = k$) of a causally related precursor event must be parallel to that of the effect itself. It must be remembered that biotransformation leading to usually less toxic, more easily excretable metabolites is most often the rate-determining step in the elimination of chemicals because processes related to excretion occur on a faster time scale. Thus, the toxicodynamic conse-

quence of biotransformation is that the AUC of elimination will be identical to the AUC of recovery. A very important exception is when a more toxic chemical is produced by an organism than the invading compound itself (metabolic activation). In this case, production of the more reactive metabolite will determine the rate of injury and hence the rate-determining step changes from elimination to the time scale of recovery from the injury. Similar considerations need to be made for the interaction between Phase I and Phase II metabolism regarding detoxification and toxification.

Distribution. This is the process when a compound is transferred from the systemic circulation into tissues. With regard to tissues, this amounts to absorption into them. Distribution can be rate-limiting, but seldom rate-determining. Almost all chemicals eventually get eliminated, which sometimes represents the slowest, and hence rate-determining, process (e.g., biotransformation). Therefore, chronic effects (when driven by *K*) will occur according to laws ruled by the terminal half-life of chemicals. However, in instances when the effect occurs during the distribution phase of a chemical, its dynamics will be determined by the distribution half-life rather than its terminal half-life. An example is chloroacetic acid-induced coma and death.

Toxicodynamics *[D = f(I,R,)]*

Toxicodynamics deals with the quantitative description of the answer to the question "What does a particular chemical do to an organism?" Although *K* has been dominated by studies of its time-dependence, *D* has been overwhelmingly dominated by dose-response studies, which have ignored time whenever possible. This was probably the reason for a lack of conceptualization of time as a variable of toxicodynamics. *D* might be viewed as the dynamic (therefore time-dependent) equilibrium between the occurrence of injury and recovery from it. At steady state, the rate of injury equals the rate of recovery. Whichever is the rate-determining step will rule the dynamics of this equilibrium similar to toxicokinetics, where elimination most often represents the rate-determining step. In toxicodynamics, recovery is most frequently found to be rate-determining. It is not common practice to use the concept of AUC in *D*, although often the toxicodynamic AUC will be the only rate-determining process in the manifestation of toxicity. This is perhaps the most neglected area of toxicology, as shown by the scarcity of data on recovery from injury. The reason for this is probably that toxicologists focused mainly on the process of injury, including its time course, although injury is much less often rate-determining than recovery because the time course of injury is usually (much) faster than that of recovery.

Injury *[I = f(S,M)]*

Understandably, but also regrettably, injury is the most widely studied part of toxicity, because much, if not most, of the time, the process of setting an injury is very rapid and hence not rate-determining. Recovery is usually slower, making it most often the rate-determining step. The definition of toxicity as the accumulation of injury (occurring usually, but not always, according to the dynamic time scale of recovery) to the point when it becomes incompatible with life indicates that death is the ultimate endpoint of all toxic effects. The important question is whether or not a particular toxic insult will or will not be rate-determining in the incapacitation and ultimate demise of an organism. There are extremely rapid rates of accumulation of injury, particularly when the process of recovery is very inefficient (hydrogen cyanide). There are also exceedingly slow rates of accumulation of injury, as in the formation of precursor lesions to cancer, when a particular form of recovery (repair) is very efficient.

Cancer caused by initiators might be viewed as being driven by dynamics because the rate of producing viable mutated cells is much slower than the repair of the DNA damage, which is often very efficient. It takes considerable time to accumulate a critical number of viable mutated cells to have a finite probability that some stimulatory or inhibitory signal will trigger sustained proliferation of such cells. In contrast, promoters do not act by a dynamic, but rather a kinetic, rate-determining step. They do not bind to DNA, but due to their long half-life (dioxins) or continuous exposure (phorbol esters), they alter for a prolonged period of time hormonal constellations or signal transduction pathways, which increase the probability of triggering proliferation of fewer initiated cells (background initiation). Thus, dynamically acting chemicals (initiators) increase the number of initiated cells, whereas kinetically acting compounds (promoters) provide a more favorable environment for cell proliferation.

Injury can theoretically accumulate linearly when there is no recovery. However, this is very seldom the case, because it only happens when the organism dies, before any recovery could occur. For example, the recovery half-life from arsine-induced hemolysis will be identical to the production half-life of erythrocytes. At very high doses, when time to death will be shorter than a fraction of the recovery half-life, injury will accumulate linearly (zero-order process). However, at lower doses, when animals do not die, hemolysis will reach steady state between injury and recovery, and if the dosing interval is within 100 days, chronic consequences might ensue.

Death

One of the major problems in dealing with toxicity is that death of an individual usually does not occur under conditions of a toxicodynamic or a toxicokinetically determined toxicodynamic steady state, but at any place along ascending or descending exponential functions of injury or recovery. This makes it essentially impossible to recognize any patterns other than under ideal conditions, when $c \times t$ becomes constant.

Death is the ultimate end-point of toxicity, because the question is always if an insult to the organism contributes to reducing the natural life-span of that individual. The answer is only if the insult becomes rate-determining or rate-limiting. But, it must be emphasized that one, or many, exposure episodes will remain inconsequential if $c_{\text{threshold}} \times t^{x}_{\text{lifespan}} = k$ is not exceeded.

Incapacitation

This is a precursor condition to death, best exemplified by the parallel dose-responses for the various stages of anesthesia (Storm and Rozman 1998). However, all toxic insults lead to incapacitation before death occurs. Recovery from incapacitation can occur without adverse residual effects according to the rate-determining steps of processes involved in recovery. However, in the worst case scenario, irreversible damage can occur, which then will become the rate-determining step.

Residual damage

A great deal is known about residual damage. For conceptual purposes it is important to consider residual damage that relates to toxicity and residual damage that is coincidental. For example, organophosphates deplete acetylcholinesterase (AChE) in the brain and in erythrocytes. Erythrocytes cannot synthesize AChE and, therefore, residual damage will persist according to the dynamics of erythrocyte production. Thus, organophosphate exposure to toxic levels can be seen for up to 60 days, and possibly longer, after exposure when determining AChE in erythrocytes (Sidell 1974). Therefore, if soldiers are

deployed for 30 to 90 days, exposure to organophosphates could be quantitated, if pre- and post-deployment blood samples were available, even though this effect has no toxicological relevance other than as marker of exposure. One major advantage of drawing pre- and post-deployment blood samples from the same individual would be an elimination of the considerable interindividual variability in terms of AChE. Some toxicities of organophosphates are due to depletion of AChE at neuronal or neuromuscular junctions, where release subsequent to synthesis of AChE is only a matter of time. Therefore, toxicity will depend on whether the rate of deactivation (covalent binding) or synthesis of new enzymes represents a rate-determining step. Usually, degradation of enzymes is slower than synthesis of new enzymes. However, binding of reactive organophosphates, such as soman to serine residues, occurs on a time scale of minutes as compared with normal degradation of enzymes, which proceed on a time scale of longer than a day. Thus, in such instances, synthesis of new enzymes, rather than their degradation, becomes rate-determining for recovery.

However, it should be kept in mind that soman-induced seizure is thought to occur in animals with rapid accumulation of acetylcholine (ACh) in the synaptic junctions. But, this is only possible if inactivation of AChE is still faster. Nevertheless, the rate-determining step in this case will become irreversible injury of the central nervous system (CNS) due to hypoxia during seizure, and not depletion of AChE. On the other hand, respiratory failure is thought to be (mainly) due to inhibition of AChE in the respiratory center. In this case, recovery (synthesis of new enzymes) will become rate-determining. Individuals survive on artificial respiration with no residual damage. Many phenomena of adaptation are related to this interplay between injury and recovery, particularly to the switch in which one, or the other, is becoming rate-determining.

Residual damage can be due to toxicokinetics, but most frequently it is related to a lack of complete recovery or the lack of any recovery. In any event, the recovery half-lives of causally related effects must be the same. Thus, if the recovery half-life from soman intoxication is 12 to 24 h (estimated from Sterri et al. 1980) and the recovery half-life of brain AChE is more than a week (Tripathi and Dewey 1989), then the two are not directly related, although, theoretically, a small amount of newly synthesized enzyme could initiate recovery.

Recovery *[R = f(Adp, Rp, Rv)]*

Most unfortunately, recovery is not a well studied part of the toxicity of chemicals. Pharmacologists routinely examine the reversibility of receptor binding, although its time-dependence is usually "hidden" in affinity constants. This author has seen very few studies in which the time course of recovery from an injury was systematically studied. Of course, there are standard protocols to study recovery (most often called reversibility) after subchronic exposure. Usually one single time point is chosen after cessation of exposure, which does not permit any kind of quantitative analysis of the dynamics of recovery. It is also clear that there are formidable experimental difficulties in investigating recovery in all or none type effects, because of aging and the limits imposed by life-span in chronic studies. No matter how much the injury caused by a chemical is studied, if its recovery half-life is much longer than the injury half-life, then the latter will dominate the dynamics of the overall process of toxicity. Another important question is whether toxicokinetics or toxicodynamics will play a rate-determining role in the recovery process. If it is toxicokinetics, the recovery process will be determined by the elimination of the compound from the organism. This is the case with dioxins and, indeed, all their effects occur according to $c \times t$ even after acute oral exposure. However, if recovery is slower than the kinetic half-life of a compound, then the recovery process will dominate toxicity. For example, clearance of aniline from blood of rats is very rapid after cessation of inhala-

tion exposure. In agreement with this, Kim and Carlson (1986) found no accumulation of aniline in blood of rats after 8- or 12-h inhalation shifts for 5 and 4 days, respectively. However, methemoglobinemia accumulated to steady state after 12-h shifts, but not after 8-h shifts. This is compatible with the slower recovery half-life of methemoglobinemia (about 3 h). After 12-h shifts, 4 recovery half-lives elapse between exposures (about 90% clearance), whereas after 8-h shifts, 5.3 recovery half-lives pass by before the next exposure (about 97% clearance), which explains why the accumulation is measurable after 12-h, but not after 8-h, shifts of exposure. This demonstrates that accumulation of damage (methemoglobinemia) to a steady state occurs according to the recovery half-life of methemoglobinemia and not according the half-life of aniline (Kim and Carlson 1986).

Adaptation

Adaptation is a well-known phenomenon, but also controversial, probably because it has not been studied nearly as thoroughly as injury. There are strong indications that adaptation might be the rate-determining effect in radiation hormesis (e.g., Caratero et al. 1998; Hoel and Li 1998). When adaptation is not rate-determining, but rate-limiting, its investigation is more difficult. The only way that appears feasible is to study recovery very carefully and to plot the recovery data to determine whether or not the process of recovery is monophasic or multiphasic (biphasic). Curve-stripping would then yield a slope for adaptation. This author is not aware of any studies having performed such analysis.

Repair

Repair is a similarly neglected field, although much more information is available on repair than on adaptation. It is not the purpose of this analysis to do an exhaustive search of the literature for papers dealing with tissue repair, but to illustrate that whenever repair is the rate-determining (slowest) process, then repair rather than injury needs to be studied. This has been done extensively for DNA repair (Pitot and Dragan 1996). A semiquantitative relationship between the half-life of adducts and carcinogenic potency has been recognized. Unfortunately, the time-course studies were less than ideally designed from the point of view of dynamics. Nevertheless, some of the better studies provide support for the concepts presented here (Swenberg et al. 1985). The significance of tissue repair or the lack thereof in the manifestation of toxicity has been recognized (Mehendele 1995), but few people study this important phenomenon other than Mehendele's group.

Reversibility

When an effect is reversed, it ends the action of a chemical in an organism. This can occur simultaneously with the elimination of a compound or with a lag period (equal to the toxicodynamic half-life), which is a measure of the reversibility or irreversibility of an effect. Considering how central reversibility is to toxicology, it is a neglected field that has been paid much less attention than the process of injury itself. Perhaps due to the fact that the majority of effects is mediated by receptors, reversibility is reasonably well understood in pharmacology (Lauffenburger and Linderman 1993). However, reversibility is much more difficult and sometimes impossible (when organisms die) to study in toxicology. This is no excuse for the relative paucity of quantitative data in terms of reversibility half-lives of effects. Even when such data are available (Swenberg et al. 1985), the experiments have not been ideally designed; this entails defining the reversibility plot for about 7 reversibility half-lives to obtain the terminal slope (if reversibility is bi- or multiphasic). The lack of quantitative information on

reversibility is very disturbing, because this will most frequently be the rate-determining step when compounds act by toxicodynamic mechanisms.

CONCLUSIONS

If there is no exposure, there is no toxicity. If there is exposure, toxicity might ensue above a certain dose and time, a topic discussed under toxicokinetics and toxicodynamics. Analysis of the fundamental equation of toxicity (Figure 1) yielded the recognition of three independent time scales. One is the dynamic time scale which is an intrinsic property of a given compound (what does a chemical do to an organism), and the other is the kinetic time scale which is an intrinsic property of a specific organism (what does an organism do to a chemical). The frequency of exposure denotes a third time scale, which is independent of dose and of the dynamic and kinetic time scales. Frequency of exposure depends on the experimentalist or nature, but not on an organism or substance. A liminal condition occurs when the frequency becomes infinite, which corresponds to continuous exposure. Continuous exposure forces the dynamic and kinetic time scales to become synchronized, thereby reducing complexity to three variables: dose, effect, and one time scale. Keeping one of those variables constant allows one to study the other variables reproducibly under isoeffective, isodosic, or isotemporal conditions. However, any departure from continuous exposure will introduce the full complexity of four independent variables (dose and the kinetic, dynamic, and frequency time scales) impacting on the effect (dependent variable) at the same time. The examples discussed here demonstrate how nature in the form of long half-lives provides liminal conditions when either kinetic or dynamic half-lives force synchronization of all three time scales.

The original charge for this paper was to conceptualize the role of toxicokinetics in the risk assessment of deployed forces exposed to chemicals. Most toxicologists familiar with current trends in toxicology are aware of the tremendous proliferation of publications combining physiologically based pharmacokinetic (PBPK) models with various dose-response extrapolation models, usually with the linearized multistage (LMS) model or more recently with the benchmark (BM) curve-fitting model. This author has used both PBPK and classical pharmacokinetics to model numerous experiments (Scheufler and Rozman 1984; Roth et al. 1994; Roth et al. 1995). Although both are conceptually sound, there is one fundamental difference: classical pharmacokinetics uses time as an explicit function, whereas PBPK deals with time as a predicted variable based on partition coefficients, tissue volumina, and blood flow rates. Therefore, concepts of classical pharmacokinetics were helpful in the development of the initial core of a theory of toxicology, as presented in this document, whereas the concepts of PBPK were not as useful. This is not to say that combining PBPK with a theoretically sound biological model will not provide appropriate answers in some instances. However, as long as PBPK is used in conjunction with biologically implausible models (LMS, BM), it will lead (not surprisingly) to insignificant improvements (Storm and Rozman, 1997; Storm and Rozman 1998). Central to the development of the concepts presented here was the notion that time is a variable equivalent to dose in toxicology. This idea has been around among toxicologists for almost exactly 100 years (Warren 1900). Nevertheless, claims of exceptions to this idea as embodied in Haber's Rule prevented the development of time as a variable in toxicology. Even today toxicologists tend to focus on the so-called "exceptions" when effects are overwhelmingly dose—but not time—dependent. They do not realize that they are studying extreme parts of a spectrum under liminal conditions (e.g., a highly reversible effect on a short time scale), and they use experimental models with insufficient time resolution. When time resolution is satisfactory (such as pungency on a scale of seconds), clear summation effects emerge (Cometto-Muñiz and Cain 1984).

Thinking about how to fix current risk assessments made a paradox clear: risk projections using elaborate mathematical models do not include time as a variable even though any and all risk predictions are by definition made in time. From this recognition it was concluded that something that is basically flawed cannot be fixed. Therefore, a new risk-assessment paradigm that includes time as a variable of toxicity is being suggested. It is clear that although dose is a simple function (number of molecules), time is a complex variable, which runs on many different scales, at least three of which are interacting with dose to provide the enormous complexity that seemed to have bewildered generations of toxicologists. The three time scales are the toxicokinetic and toxicodynamic half lives and the frequency of exposure. Thus, there are three liminal conditions:

1. When the toxicokinetic half-life is very long, it keeps the frequency of exposure constant (continuous exposure), and the toxicodynamic half-life by definition will be the same as the toxicokinetic one. Under these liminal conditions $c \times t = k$ for isoeffective experiments, because there is only dose-dependence and one time-dependence.
2. When the toxicodynamic half-life is very long, it requires no additional injury to occur to keep injury constant nor the continuous presence of the noxious agent to result under isoeffective conditions in $c \times t = k$, because there is only dose-dependence and one time-dependence.
3. When the toxicokinetic/toxicodynamic half-lives become very short, they will blur the distinction between the kinetic and dynamic time scales and both will become less important, because in that case the frequency of exposure dominates the time-dependence. Under liminal (continuous exposure = infinite frequency) and isoeffective conditions, this will also lead to $c \times t = k$.

When experiments are conducted under isodosic or isotemporal conditions then the relationship will obey the equation $c \times t = k \times \textit{Effect}$ (Figure 4). The vast majority of exposure scenarios are of course far from these liminal situations (ideal conditions) and will, therefore, yield $c \times t^x = k$. There are clear suggestions in this paper for the type of experiments that need to be done to determine x with exactitude. In the meantime, practical suggestions illustrate how to use a decision tree or available databases to conduct less arbitrary risk assessments than currently done for any conceivable deployment situation by using the new risk paradigm, which includes both dose and time as variables of toxicity.

To Summarize:

1. The charge was to conceptualize the role of kinetics in risk assessment. The current trend is to use PBPK rather than classical kinetics. The author prefers classical kinetics, because time is an explicit variable there.
2. Adding time to dose as an independent variable in toxicology allows a new risk assessment paradigm, which does not depend on defaults and uncertainties associated with current methodology (reference dose, linear multistage extrapolation, etc.) in which the only independent variable is dose.
3. A decision-tree approach is outlined in Appendix 1 in which the first step is the identification of the target species and chemical and the specific adverse effect to be applied to the deployment scenario. The second step involves the use of the kinetic and dynamic information relative to this scenario to define the rate-limiting or rate-determining steps. The final step is to use the kinetic or dynamic half-life, together with the anticipated schedules, to predict exposure thresholds for the deployed forces.
4. Examples are provided to illustrate various liminal conditions where $c \times t = k$ (very long kinetic or dynamic half-lives versus very short kinetic or dynamic half-lives where exposure frequency becomes the limiting condition). Examples are also provided for the more common exposure situation where $c \times t^x = k$ as well as the methodology for risk predictions.

5. A somewhat simplified approach using occupational exposure limits as the starting point is described in Appendix 2, together with examples of how this approach could be used to protect deployed forces.

APPENDIX 1

Examples to Illustrate the Use of the Decision Tree Concept in Risk Analysis

Dioxin (TCDD)

Toxicodynamics

half-life unknown, but unlikely to be rate-determining

Injury
- Death: wasting, hemorrhage, anemia, cancer
- Incapacitation: little
- Residual damage: chloracne (human)
  multiple (animals)

Recovery
- Adaptation
  - Pretreatment: induction of enzymes (ineffective)
  - Exercise: unlikely
  - Stress: unlikely
  - Temperature: unlikely
- Repair
  - DNA repair: no DNA damage
  - Apoptosis: some
  - Cell replication: yes
- Reversibility
  - Receptor binding: strong
  - Chemical bonding: no
  - Hydrogen bonding: no
  - Ion-Ion interaction: no

Toxicokinetics (Figure 6)

half-life 7 years (humans)
half-life 20 days (rats)
This will be the rate-determining process.

Absorption
- Oral: 90%
- Inhalation: negligible
- Dermal: negligible
- Other: negligible

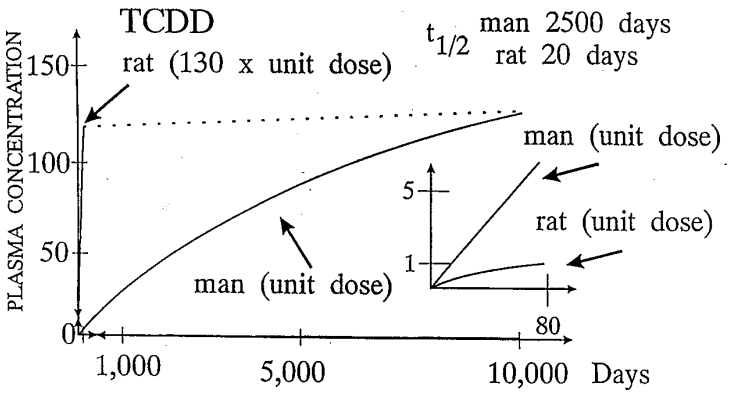

FIGURE 6 Schematic illustration of the effect of different half-lives of TCDD on steady-state concentrations in humans and rats given the same daily dose rates.

Elimination
- Distribution
  - Adipose tissue: sequestration
  - Liver protein: sequestration
  - Bone matrix: negligible
- Biotransformation
  - Phase I: rapid, very little
  - Phase II: rapid, but not much
  - Polymorphism: unimportant because of lack of biotransformation
- Excretion
  - Renal: negligible
  - Fecal: slow (rate-determining)
  - Biliary/enterohepatic circulation: very little

Minor routes: negligible

Risk Assessment for Dioxin

It takes 6.64 half-lives (46.5 years) to eliminate 99% of a single dose of TCDD. TCDD will accumulate and reach 99% of steady state after 6.64 half-lives (46.5 years) following repeated exposure.

Thus, x, of the $c \times t^x$ term, will be very close to unity for this compound, implying very little margin of safety above the $c \times t$ lifetime threshold. This, combined with the lack of possibility for toxicokinetic intervention (which is the rate-determining step), makes it mandatory that the source of exposure must be controlled, which is the food.

Because toxicokinetics is the rate-determining step in the toxicity of dioxins, interpretation of animal data has to take into account the large difference in half-lives between humans (half-life = 7 years) and laboratory animals (half-life = 20 days). If $c \times t$ estimates are derived from rat data, they have to be divided by 128 to ascertain that the AUC will not be larger in humans in 46.5 years than in rats after 133 days (Figure 6).

These types of compounds will be detectable in exposed people for the rest of their lives. Moreover, the magnitude of their exposure can be extrapolated back in time with just a few assumptions about exposure scenarios. The accuracy of these projections is very high.

During 1- to 30-day deployments, it will not matter much in terms of risk assessment if exposure will be due to a single hostile act or to insidious repeated exposures.

It can be derived from the $c \times t = k$ relationship (Table 1) that the lifetime lowest- observed-adverse-effect level (LOAEL) for HpCDD-induced lung cancer in female rats with a life expectancy of 1,000 days would be a dose of 1,180 µg/kg. Because the difference between LD_{100} and LD_0 is a factor of 2, virtually all female rats will be protected from lung cancer by a dose of 590 µg/kg of HpCDD. In terms of daily dose rate, this corresponds to 0.59 µg/kg/day. Due to the long half-life of HpCDD (314 days in female rats), exposure would be continuous and therefore $x = 1$ (approximately) for all biological endpoints. Thus, any dose of HpCDD that would not cause minimum enzyme induction (ethoxyresorufin-o-deethylase [erod]) would represent a margin of safety (*MS*) that would correspond to a 59-fold margin of safety or a 1.7×10^{-2} margin of risk.

$$MS = \frac{c \times t_{\text{lungcancer}}}{c \times t_{\text{erod}}}$$

Conversion of these margins for humans would require an adjustment for the longer life expectancy of humans (25,550 days versus 1,000 days) under an assumption of equal sensitivity, resulting in a 2.3-fold margin of safety or a 4×10^{-1} margin of risk for humans at this dose. It is a risk management decision to reduce these numbers by arbitrary safety factors. However, because exposure has been reduced to subthreshold levels, no additional safety will be gained by the application of large safety factors. As the relative potency of dioxins to cause cancer (Rozman et al. 1993) is the same as that to cause other effects, these calculations can also be applied to other dioxins to calculate the margins of safety and risk. For tetra-dioxin (toxic equivalent factor [TEF] of TCDD [1] versus HpCDD [0.007]), this calculation yields a daily lifetime dose rate of 4.1 ng/kg. Assuming that risk is linearly decreasing, also below the threshold, this calculation would yield a risk of 0.4×10^{-6} for a daily exposure to 4.1 ng/kg of TCDD. According to the $c \times t$ concept, lung cancer could occur in a sensitive human being after 10,250,000 days, or 28,082 years, of exposure to TCDD at this daily dose rate.

Soman

Toxicokinetics

half-life ≅ 10 to 100 min in animals (Benschop and De Jong, 1991)
half-life unknown in humans (unlikely to be much different from animals)
↓
not rate-determining

Toxicodynamics

half-life ≅ 12 h (animals)
half-life unknown (humans) (unlikely to be much different from animals)

Injury: very rapid (not rate-determining)
Death: cholinergic crisis/hypoxia

Incapacitation: cholinergic crisis
Residual damage: CNS/hypoxia
Recovery: slow (rate-determining)
half-life ≅ 12 h (Sterri et al. 1980)
Synthesis of new enzyme at critical sites (CNS)
Adaptation: limited
Pretreatment:
Pretreatment with soman does not change its toxicity
Pretreatment with agents, which bind to the same site reversibly provides possibility for therapeutic intervention
Exercise:
Increases rate of respiration and therefore will lead to higher systemic exposure
Stress:
Same as for exercise
Temperature:
There is a temperature effect
Repair
DNA repair: not critical
Apoptosis: not critical
Cell proliferation: coincidental impact
Synthesis of new biological entity: slow (rate-determining)

Cell Proliferation:

This is not a critical step in the assessment of soman toxicity. However, it is important in the assessment of exposure to soman. Soman binds promiscuously to all sites capable of nucleophilic displacement of flouride (Fl^-), which includes erythrocytes. Although this in itself does not have toxic consequences, it produces a rate-determining step (production of new erythrocytes) that allows an exposure assessment much longer than the rate-determining step of toxicity (synthesis of new acetylcholinesterase [AChE] in CNS). If both pre- and post-deployment blood samples were available, significant exposure to anticholinesterase compounds could be demonstrated for up to 90 days after an acute, subchronic, or chronic intoxication situation. This method would be somewhat nonspecific and time-dependent because all acetylcholinesterase inhibitors, including organophosphate and carbamate pesticide, would also temporarily inhibit AChE.

Synthesis of a biochemical entity:

It appears from the literature that the rate-determining step in soman intoxications is recovery in the form of synthesis of new AChE in the CNS, specifically in the respiratory center (Lintern et al. 1998). Injury (binding to AChE) is very rapid and hence not rate-determining. The estimated half-life of recovery is about 12 h (Sterri et al. 1980), which is in good agreement with the half-life of synthesis of some enzymes. This represents a reversal of the normal situation when degradation of a protein is the rate-determining step in maintaining a steady-state concentration of an enzyme. This is the therapeutic window when reversibly binding agents can protect newly synthesized AChE from irreversible reaction with soman before natural degradation becomes rate-limiting again. As useful as these mechanistic considerations might be for the development of therapeutic intervention, knowledge of the mechanism of toxicity is not needed for risk assessment. What is essential though, is identification of the rate-determining step and its modeling to obtain a half-life of that process.

Risk Assessment for Soman

Single dose, subcutaneous exposure:

$c \times t$ = 17,858 µg/kg/min (Sivam et al. 1984)

17,858 / (24 × 60) = 12.4 µg/kg/day

This is valid if exposure is continuous and effect entirely irreversible.

Primates are about 10 times more sensitive, because of the lower levels of the nonspecific esterases (as a detoxification sink) (Anzueto et al. 1986; 1990)

12.4/10 = 1.24 µg/kg/day

Primates are very similar to each other, including humans (Talbot et al. 1988)

The dose response for soman is extremely steep, at most a factor of 2

1.24/2 = 0.62 µg/kg/day (Aas et al. 1985)

Recovery half-life from soman intoxication is estimated at 12 h (Sterri et al. 1980; Lintern et al. 1998)

Therefore, if exposure occurs outside of 6.64 recovery half-lives, there will be no accumulation of injury.

If exposure occurs within 6.64 recovery half-lives, there will be accumulation of injury to steady state after 6.64 half-lives.

As a first approximation, the daily reference dose needs to be divided further by the number of days exposure occurred.

Oral

1-day deployment exposure limit (DEL) by ingestion: 0.62 µg/kg/day

14-day DEL by ingestion: 0.04 µg/kg/day

30-day DEL by ingestion: 0.02 µg/kg/day

These are probably very conservative estimates because organophosphates have strong hepatic first-pass effect and these numbers were derived from subcutaneous injection, which is kinetically closer to inhalation than ingestion. This notion is supported by the similarity of the $c \times t$ product after inhalation and subcutaneous injection.

Inhalation (Aas et al. 1985)

$c \times t$ = 520 mg/m^3/min at a concentration of 21 mg/m^3

t = 24.8 min

Rats (~150 to 200) inhale about 500-600 ml air/min (Druckrey et al. 1967)

According to calculations using metabolic body size and caloric utilization (Kleiber, 1975; Kleiber, 1975), rats of this size should have a breathing rate of 481 ml/min, which is close to Druckrey's estimate.

Converting inhalation to oral dose:

500 ml/min air inhaled for 24.8 min → 12,400 ml → 12.41 → 0.0124 m^3

at 21 mg/m^3 → 0.2604 mg/rat for a 260 g rat → 1.002 mg/kg = 1,002 g/kg

$c \times t$ = 24,848 g/kg/min

This is very similar to the subcutaneous $c \times t$ (17,858 µg/kg/min).

$c \times t$ = 520 mg/m^3/min

Primates 10 times more sensitive than rats (Anzueto et al. 1990)

$c \times t$ = 52 mg/m^3 /min

Steep dose response of factor 2

$c \times t$ = 26 mg/m^3/min (LOEL)

$c \times t$ = 433 µg/m^3/h

$c \times t$ = 18.0 µg/m^3/day

Breathing-rate conversion of rat to human's ~ 3.7

$c \times t = 66.6\ \mu g/m^3/day$

Because this represents an estimated lowest-observable-effect level (LOEL), risk managers might divide it by a safety factor of 10 to ascertain that no one will be exposed to near toxic concentrations of soman.

1-day deployment exposure limit (DEL): 7 $\mu g/m^3$
14-day DEL: 0.5 $\mu g/m^3$
30-day DEL: 0.2 $\mu g/m^3$

These values are DELs for continuous exposure. If exposure is intermittent, the following rule will provide protection: If exposure occurs outside of 6.62 recovery half-lives (3.3 days at a half-life of ~ 12 h) the 1-day DEL will provide protection for all scenarios, because there will be no accumulation of injury.

The 1-day DEL should not be exceeded if exposure occurs for shorter durations (e.g., 1 h or 10 min), because in 24 h there will be only 75% recovery and injury will proceed to steady state, and after 3.32 half-lives it will be accumulating according to $c \times t = k$.

APPENDIX 2

Strategies for Developing Exposure Guidelines of Deployed Forces

Development of exposure guidelines from existing databases (ACGIH, NIOSH, OSHA):

(1) Identify if compound exerts its rate-determining step(s) by toxicodynamic or toxicokinetic means (Figure 1).

(2) Narrow down critical step(s) to injury/absorption or elimination/recovery or further to adaptation/distribution, repair/biotransformation, and reversibility/excretion.

(3) Estimate half-life of critical step(s).

This is a very important step. Failure to identify the proper process and its half-life can lead to erroneous conclusions. This can be illustrated with a pharmacological example to indicate the universality of this approach.

Omperazole is a H^+ -ion pump inhibitor. The binding half-life to its receptor is 24 h. Its biological half-life is about 1 h. If the therapeutic dose would be based on the biological half-life of the compound, the conclusion would be that after 6.64 half-lives (6.64 h) 99% of the drug would be eliminated. Therefore, a dosing regiment entailing the administration of the drug every 6 h still would yield a very poor steady state and, with that, moderate to low therapeutic efficacy. Omperazole in fact is given daily once or every other day in accordance with its pharmacodynamic half-life of 24 h. It has been reported that it takes 3 days for maximum effect (clinically indistinguishable from 3.32 half-lives) and 3 to 5 days for cessation of effect, which is clinically also indistinguishable from 3.32 reversibility half-lives. The action of this drug is entirely dominated by *D* and any opinion, based on *K*, would be in error.

(4) Because threshold limit values (TLVs), short-term exposure limits (STELs) and ceilings (ACGIH), permissible exposure limit (PELs) (OSHA), and recommended exposure levels (RELs) (NIOSH) represent NOAELs, one could use these values and convert them to any given deployment situation by considering duration and frequency of exposure along with the half-life of the critical step, which could be designated as exposure kinetics (see equation in Figure 1). A twofold critical question is: When are time-weighted averages (TWAs) and when are peak concentrations important for converting these standards into standards for various deployment situations?

Derivation of DEL When Toxicokinetics Is Rate-Determining

Very long toxicokinetic half-lives

Compounds having half-lives of years or longer (e.g., mirex, dioxins, cadmium, asbestos) are the simplest to deal with. (It is amazing that regulatory agencies have been struggling with this for decades.)

For such compounds, any $c \times t$ conversion will be fairly accurate without regard to route and frequency of exposure. A single high dose exposure will not be much different from exposure to proportionally smaller daily dose rates. Thus, for these types of compounds, there is little difference between TWA and peak exposure.

Example: *cadmium* half-life ≅ 30 years (in humans)

Inhalation
TLV (ACGIH): 0.01 mg/m^3, 8-h TWA for 5 days/week for 45 years at 10 m^3/8 h
Total dose: 1,170 mg/70 kg person
16.7 mg/kg
1-day limit by inhalation: 1,170 mg/20 m^3 = 58.5 mg/m^3 at 20 m^3 (air inhaled/24 h)
Similar to radiation, this is a ceiling value for total exposure, which might not be exceeded. Persons must be protected from further exposure. It is a risk management decision to reduce this number, e.g., by dividing it by 10.

1-day deployment exposure limit (DEL) by inhalation: 5.9 mg/m^3
14-day DEL by inhalation: 0.4 mg/m^3
3-month DEL by inhalation: 0.2 mg/m^3

Oral
Conversion of inhalation to oral dose:
20 m^3 × 5.9 mg/m^3 ÷ 70 kg = 1.69 mg/kg
Absorption of cadmium salts in the GI tract is very limited at 5 to 8% of dose (Goyer 1996), which provides additional safety.
1-day DEL by ingestion: 1.69 mg/kg
14-day DEL by ingestion: 0.12 mg/kg/day
3-month DEL by ingestion: 0.06 mg/kg/day

It is a risk management decision to reduce these numbers to leave room for reserve capacity, although the safety factor does not have to be large, because of low fractional absorption, which reduces systemic exposure by nearly a factor of a 100.

Dermal
Dermal penetration of cadmium salts and of highly lipophilic compounds of very long half-life is very inefficient.

Intermediate toxicokinetic half-lives

For compounds having a half-life 3.6 h or longer, but shorter than months or years, elimination will not be complete within 24 h after a single dose. This is important, because, as a consequence, recovery from injury will also not be complete. Recovery from injury by chemicals having intermediate half-lives will be probably as often rate-determining (rate-limiting) as the biological half-

life. Therefore, a discussion of these types of chemicals will be also found in the toxicodynamic section as well. For such compounds, peak exposures as well as TWA concentrations will be of consequence. Therefore, particular attention must be paid to the ratio between the biological half-life and various exposure scenarios when setting DELs for such chemicals. It is worthwhile to consider that the half-life of 3.6 h represents among this class of compounds the best case scenario, because the longer the half-life, the higher the accumulation will be by the time a steady state is reached.

Example: *monochloroacetic acid (MCA)*

half-life $\cong$ 4 h (rats)

The half-life is probably very similar in humans because the mechanism of toxicity is the same and the rat's LD_{50} = 75 mg/kg is similar to the human's lethal dose $\cong$ 220 mg/kg.

Inhalation

TLV recommendation: 2 mg/m^3, 8-h TWA for 5 days/week for 45 years at 10 m^3/8 h

Total dose: 234,000 mg/70 kg person

3,343 mg/kg

1-day DEL by inhalation: 1 mg/m^3 at 20 m^3/24 h

14-day DEL by inhalation: 0.07 mg/m^3

3-month DEL by inhalation: 0.03 mg/m^3

This is the most conservative conversion, assuming continuous exposure. A comparison of the 1-day total DEL (20 mg) with the total occupational exposure limit (OEL) in 45 years (234,000 mg) indicates how sensitive these type of compounds are to the frequency of exposure.

After 6.64 half-lives, 99% of steady state will be reached. The half-life of MCA is about 4 h, and steady-state concentration will be reached after about 1 day. Thereafter, the AUC will increase according to $c \times t$ (above the lifetime threshold) if exposure is uninterrupted.

However, if exposure is intermittent, say for 1 h every day, then higher levels might be tolerated without adverse effect, because complete (99%) elimination/recovery will occur between any two episodes of exposure.

1-hour DEL by inhalation: 16 mg/m^3 at 1.25 m^3/h air inhaled

Oral

Assuming six (three eating and drinking and three additional drinking) intake episodes per day with a half-life of 4 h, almost 99% of steady state will be reached after 1 day. Continuous exposure for 14 or 30 days will be occurring with smaller or larger fluctuations between $C_{\text{maximum serum contration}}$ and $C_{\text{minimum serum concentration}}$ if eating/drinking are not equally paced, which will not effect the average AUC. Therefore, safe levels can be calculated for oral exposure according to $c \times t = k$ also for this compound.

Conversion of inhalation dose to oral dose:

1 mg/m^3 $\times$ 20 m^3 (air inhaled/ 24 h) = 20 mg/70 kg person = 0.3 mg/kg

The same (cumulative) dose can be ingested safely during 14 or 30 days of deployment according to the most conservative conversion, which assumes repeated exposures every day.

1-day DEL by ingestion: 0.3 mg/kg

14-day DEL by ingestion: 0.02 mg/kg/day

30-day DEL by ingestion: 0.01 mg/kg/day

Again 1-h ingestion (single daily exposure) might be higher since there will be 99% elimination/recovery within 24 h.

1-hour DEL by ingestion: 7.1 mg/kg if no further exposure occurs on that day.

Dermal

Dermal toxicity of MCA is similar to its oral toxicity as dermal penetration of MCA is extremely efficient, particularly at higher temperatures. Therefore, the same numbers are recommended for dermal exposure as for oral exposure.

As the half-life of compounds becomes longer than about 4 h, accumulation to steady state will take correspondingly longer. Nevertheless, $c \times t$ conversions will remain conservative (but not extremely conservative and not arbitrary) estimations of safe levels of exposure, because the increase of AUC during the ascending phase of the exposure curve will be smaller than at steady state. With increasing half lives, deviations from $c \times t = k$ will become less dependent on the frequency and duration of exposure.

Very short toxicokinetic half lives

If half-lives are shorter than 3.6 h then more than 99% of the compound will be eliminated within 24 h of exposure. With such compounds, the frequency of exposure will become more and more important as the half-life decreases further, because of increasing periods of recovery between exposure episodes.

Example: *Methylene chloride (MCl)*

half-life ≅ 5 to 40 min (humans), indicating an average of 22.5 min

Inhalation

TLV: 174 mg/m^3, 8-h TWA for 5 days/week for 45 years at 10 m^3/8 h

For compounds of very short half-life, it is not meaningful to use the $c \times t$ conversion unless exposure is continuous, because of rapid elimination/recovery after cessation of exposure. Rapid elimination/recovery in turn reduces time-dependence of toxicity. Compounds having very short half-lives will be mainly concentration-dependent, particularly when considering short time scales such as 1 day, 14 days, or 30 days. In fact, toxicity of such compounds depends primarily on the time scale of frequency and duration of exposure in addition to the dose (concentration). For these reasons, an OEL of 25 to 50 parts per million (ppm) (86.8 to 174 mg/m^3) was derived for MCl from impaired flicker fusion reflex data (4-h exposure) in humans (Storm and Rozman 1998). Although this behavioral effect is highly reversible, it impairs optimum performance. Therefore, in a deployment situation these values could be used as ceilings not to be exceeded. Because these values were derived with continuous exposure, the 1-hour DEL (continuous exposure) could be set based on $c \times t$ yielding 100 to 200 ppm (348 to 696 mg/m^3). However, longer-term intermittent exposures should use the ceiling approach:

1-day DEL by inhalation: 174 mg/m^3
14-day DEL by inhalation: 174 mg/m^3
30-day DEL by inhalation: 174 mg/m^3
1-hour DEL by inhalation: 696 mg/m^3

Oral

Because of significant first-pass biotransformation, oral DELs require additional considerations, but they would be higher than those obtained for inhalation.

Derivation of DEL When Toxicodynamics is Rate-Determining

Compounds having very long recovery half-lives

There are few examples for recovery taking place on a time scale of years or longer. Damage to neurons (e.g., lead encephalopathy) is the closest example that comes to mind. Because of the enormous

reserve capacity and plasticity of the nervous system, it is difficult to conduct conclusive studies in this area. Unlike compounds with very long toxicokinetic half-lives (which amounts to continuous exposure), both the frequency and duration and the kinetics of compounds are important when dynamics of the effects are rate-determining. When the toxicokinetic half-life of a compound is long (lead), a TWA approach ($c \times t = k$) will be the best way to protect deployed forces, whereas in the case of short half-life compounds (methanol), the ceiling approach must be applied to prevent the beginning of accumulation of injury.

Example: *lead*

half-life ≅ 20 years

Inhalation

TLV: 0.05 mg/m^3, 8-hr TWA for 5 days/week for 45 years at 10 m^3/8h
Total dose: 5,850 mg
5,850 mg/20 m^3 = 293 mg/m^3
It might be advisable to reduce these numbers by a safety factor of 10.
1-day DEL: 29.3 mg/m^3
14-day DEL: 2.1 mg/m^3
30-day DEL: 1.0 mg/m^3
1-hour DEL should not significantly exceed the TWA, even if exposure remains infrequent.

Oral

Conversion of inhalation to oral dose:
20 m^3 × 29.3 mg/m^3/70 kg = 8.4 mg/kg
Adults absorb 5 to15% of ingested lead, which provides enough margin of safety.
1-day DEL: 8.4 mg/kg
14-day DEL: 0.6 mg/kg/day
30-day DEL: 0.3 mg/kg/day

Dermal

Insignificant.

Example: *methanol*

Inhalation

TLV: 200 ppm = 262.1 mg/m^3

It is not appropriate to use a TWA for methanol, because in this case accumulation of injury will be a function of the frequency of exposure above the threshold. Any exposure above the threshold will be cumulative. Therefore, to protect deployed forces from such scenarios a ceiling must be established.

1-day DEL: 262.1 mg/m^3
14-day DEL: 262.1 mg/m^3
30-day DEL: 262.1 mg/m^3

Intermediate toxicodynamic half-lives

Recovery from injury caused by compounds having a toxicodynamic half-life of 3.6 h or longer, but shorter than years, will be incomplete after 1 day. In the best-case scenario of 3.6 h, recovery will be 99% complete. However, even for such compounds, $c \times t = k$ would be running at about 1% residual damage. This might be of little consequence if exposure is intermittent, but in some instances it might

represent a hazard. A better illustration of this point can be made by using nitrosamines as examples. Nitrosamines are *strong* alkylating agents with short toxicokinetic half-lives (Druckrey 1967). However, the repair half-life of the DNA adducts is about 20 to 40 days. Consequently, the steady state of DNA damage will not be reached until about 200 days (on average). Thereafter, damage will accumulate according to $c \times t = k$. Just like a single dose of a chemical having very long toxicokinetic half-lives can cause cancer (Rozman 1999), single doses of dialkylnitrosamines can also cause cancer (Druckrey et al. 1964). If one mole (approximate LD_{50}) of dimethylnitrosamine is administered to rats, and assuming that 0.0001% of it will end up binding to DNA, this will still yield 6×10^{17} adducts. With a 30-day toxicodynamic half-life this will leave a 900-day old rat still with about 6×10^{8} adducts. It is very likely that the traditional distinction between initiators and promoters in carcinogenesis is due to the respective compounds having toxicokinetic or toxicodynamic processes as rate-determining steps in their mechanism of action.

Example: *dimethyl-or diethylnitrosamine*

TLV: This is a compound listed in the TLV booklet without a value.

Dimethylnitrosamine's carcinogenicity was studied by Druckrey et al. (1963) under isoeffective conditions (100% carcinomas). Different daily dose rates of diethylnitrosamine yielded a $c \times t = k$ equal to 73,248 ± 5,234 (SE) mg/kg/day. Druckrey et al. (1967), like everybody else, viewed the daily dose rate as dose. Therefore, they plotted the daily dose rate versus time to derive a slope, which yielded the equation $c \times t^{2.3} = k$. This erroneous view led to the introduction of the notion about the reinforcing action (200-fold) of low doses. This mistake was due to the fact that their studies were not conducted under isotemporal conditions. Therefore, some rats received on the average 68 dose rates, whereas others received up to 840 dose rates. In fact, the difference in terms of cumulative dose was only 15-fold between the highest and lowest dose, which is comparable to the difference in induction time (12-fold). Suffice to say that Druckrey et al.'s (1963) data are in fact very consistent with $c \times t = k$, when viewing the dose as the sum of all dose rates.

Derivation of DELs from the $c \times t$ data:

$c \times t$ = 73,248 ± 15,700 (SD)

Rat: $c \times t_{\text{lifespan}}$ = 73,248 mg/kg/day

Lifespan of rat: 900 days

c = 73,248/900 = 81.4 mg/kg

This is the minimum dose required to cause cancer in rats after lifetime exposure. If it was chosen to protect rats from the carcinogenic effects of diethylnitrosamine to the extent of 99.94% (3 SD), then this dose would be:

$c \times t$ = 73,248 ± 15,700 (SD)

73,248 – 47,100 = 26,148/ 900 = 29.1 mg/kg

However, humans do not live 900 days, so let us assume 75 years (27,375 days). Under assumption of equal sensitivity this would yield a minimum carcinogenic dose in humans of 26,148/ 27,375 ≅ 1 mg/kg If human in vivo repair of DNA adducts were known, this number could be adjusted quite accurately, and species differences could be dealt with in a scientific rather than arbitrary fashion.

A small safety factor might be applied by risk managers to move from a LOAEL to a NOAEL. The number should be small, because individual sensitivity was corrected for based on normal distribution rather than on arbitrary assumptions.

Inhalation

0.5 mg/kg × 70 kg = 35 mg/kg

35 mg/20 m^3 = 1.75 mg/20 m^3 (air inhaled/ 24 h)

1-day DEL: 1.75 mg/m^3
14-day DEL: 0.13 mg/m^3
30-day DEL: 0.06 mg/m^3

Oral

1-day DEL: 0.5 mg/kg
14-day DEL: 0.04 mg/kg/day
30-day DEL: 0.016 mg/kg/day

Dermal

Because it is a small amphophilic molecule, it will be readily absorbed through the skin. If needed, the oral data apply.

Very short toxicodynamic half-lives

The distinction between toxicokinetic and toxicodynamic half lives becomes fuzzy for compounds of very short recovery half-lives (<3.6 h), because for both of them another time scale (frequency of exposure) becomes the dominant time function. A very short recovery half-life implies rapid reversibility, or repair, or adaptation. Infrequent exposure to such compounds will have the least toxicological consequences, although in the case of air this could become rapidly fatal.

The efficient repair of oxygen-induced DNA repair appears to be a good example for rapid reversibility (Ames, 1989; Fraga et al. 1990). It takes continuous exposure to air over a lifetime to result in accumulation of oxidative damage in the form of aging.

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